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Perspective

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Antiviral Resistance in Influenza Viruses — Implications for Management and Pandemic Response

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The Centers for Disease Control and Prevention (CDC) recently issued an alert instructing clinicians to avoid using M2 ion-channel inhibitors (amantadine and rimantadine) during the current influenza season because amantadine resistance has been detected at an extraordinarily high frequency in isolates of influenza A (H3N2) virus (see table).[1-3] This and other reports[4] raise important questions regarding the implications of resistance to antiviral agents for the current clinical management of influenza and for planning for a possible pandemic.

Phenotypic amantadine resistance was first described soon after the drug was discovered in the early 1960s, and subsequent work has established that single point mutations in critical residues of the M2 protein confer high-level resistance to amantadine and rimantadine (see diagram), apparently without compromising the virus's ability to replicate, its pathogenicity, or its transmissibility.[2] Resistant variants have been detected at high frequencies (ranging from 30 percent to 80 percent) among isolates from patients who have been treated with amantadine, and household-based and institutional studies have demonstrated the potential of these variants to be transmitted to close contacts and to cause failures of chemoprophylaxis. However, studies of community isolates generally revealed low levels of primary resistance (approximately 1 percent to 3 percent) until 2003, when the incidence increased dramatically in China, possibly owing to increased use of over-the-counter amantadine after the emergence of severe acute respiratory syndrome (SARS). During the 2004–2005 influenza season, approximately 70 percent of the influenza-virus isolates from China and Hong Kong and nearly 15 percent of those from the United States and Europe showed resistance[2] (see table).

The high frequency of resistance among recent isolates of influenza A (H3N2) from North America appears to be attributable to an unprecedented global spread of viruses with a specific mutation (Ser31Asn), which has occurred despite the absence of sustained selective drug pressure. This observation clearly indicates that this mutation does not reduce transmissibility. Indeed, the fact that this particular mutation has been found consistently in community isolates of H3N2 where-

as other resistance mutations are uncommon, along with the fact that this mutation has not been found in high frequency in isolates of H1N1, suggests that it confers a transmission advantage, perhaps mediated by compensatory mutations in other genes, or that it has been incorporated into efficiently spreading viruses.

According to the CDC, between October 2, 2005, and January 21, 2006, 96.8 percent of the influenza viruses isolated in the United States were influenza A, and 99.5 percent of those that were characterized were H3N2. The H3N2 strain in this year's vaccine offers a good antigenic match for most isolates, but approximately 15 percent of the isolates show evidence of drift that might increase the likelihood of breakthrough illness in vaccinees. Unfortunately, the clinical consequence of M2-inhibitor resistance is that amantadine and rimantadine are now ineffective for prophylaxis against and treatment of infections caused by these viruses and will not have reliable efficacy in regions where there is substantial activity of influenza A (H3N2) or influenza B.

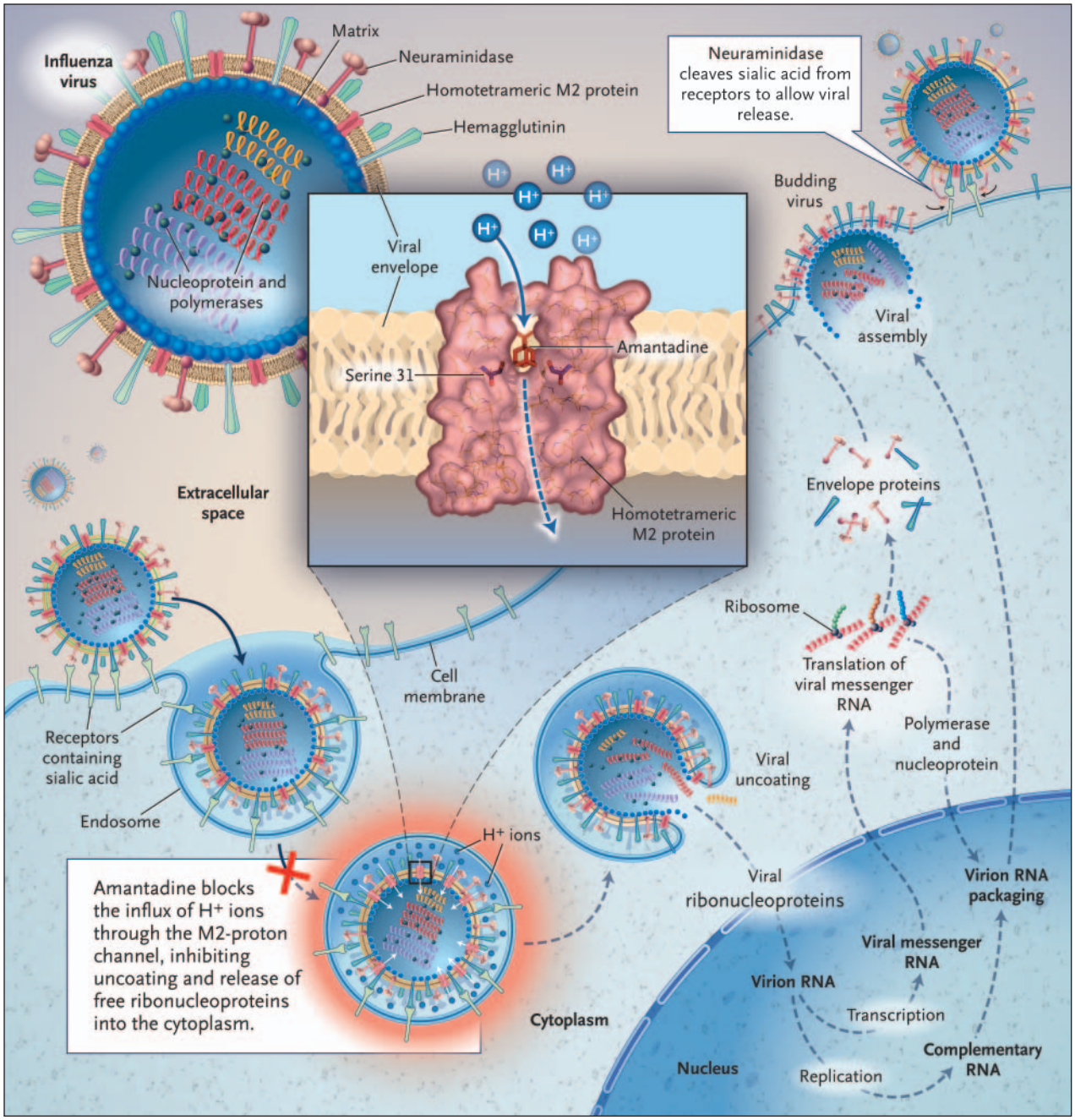
Several practical consequences follow. The neuraminidase inhibitors have now become the drugs of choice for these indications, although availability may be problematic. During last year's influenza season, approximately 1.7 million prescriptions were written for oseltamivir in the United States, and according to the manufacturer, nearly twice as much drug was produced for this year's season. Consideration could be given to using inhaled zanamivir for early treatment and prophylaxis (it is under review by the Food and Drug Administration for the latter indication), but the modest use of zanamivir in recent years has resulted in very limited availability for the current season (fewer than 40,000 courses in the United States). Third-party payers that previously covered only the less expensive M2 inhibitors will need to reimburse the prescription costs of neuraminidase inhibitors at the rates used for the lowest tier of their formulary. In order to avert shortages, physicians should heed the guidance of the World Health Organization, the Infectious Diseases Society of America, and other groups by refusing to prescribe neuraminidase inhibitors for personal stockpiling.

Does this mean that we should abandon M2 inhibitors entirely? Not yet. The M2 inhibitors proved effective for prophylaxis against influenza illness in the 1968 pandemic of "Hong Kong influenza" and in the 1977 pandemic-like event involving "Russian influenza." Although the same resistance marker (Ser31Asn) was present in two isolates of influenza A (H5N1) obtained from patients in China in 2003 and in one lineage of avian and human H5N1 viruses in Thailand, Vietnam, and Cambodia, most tested isolates from a second lineage that has been circulating recently in Indonesia, China, Mongolia, Russia, and Turkey appear to be sensitive to amantadine.[5] Furthermore, the next pandemic virus may be one that, like H2N2, is susceptible to this class of drugs. If the circulating strain were known to be susceptible to M2 inhibitors, these drugs would offer a less costly alternative to neuraminidase inhibitors for prophylaxis against illness. The 5 million courses of rimantadine currently in a U.S. federal stockpile could prove valu-

Incidence of M2-Inhibitor Resistance among Human Influenza A (H3N2) Viruses in the United States.*

Period	No. of Isolates Tested	No. That Showed Resistance (%)
1992–1995	991	8 (0.8)
1996–1997	508	2 (0.4)
1998–1999	510	11 (2.2)
2000–2001	283	4 (1.4)
2002	290	4 (1.4)
2003	174	3 (1.7)
2004	466	9 (1.9)
October 2004–March 2005	636	92 (14.5)
October–December 2005	209	193 (92.3)

* Data for 1992 through 1995 are from Ziegler et al.[1] The total includes up to 20 percent influenza A (H1N1) viruses, all of which were susceptible to M2 inhibitors. All resistant viruses were influenza A (H3N2); seven of eight resistant variants contained the Ser31Asn mutation. Data for 1998 through the October 2004–March 2005 period are from Bright et al.[2] The Ser31Asn mutation was found in 98.2 percent of the resistant variants. From 1998 through 2004, resistance was observed in 2 of 589 H1N1 viruses collected worldwide (0.3 percent; Val27Ala and Glu34Lys) and in 1 of 83 H1N2 viruses isolated in the United States (Ala30Thr). Data for October through December 2005 are from Bright et al.[3] Six H3N2 viruses with Ser31Asn also contained Val27Iso in M2; two of eight H1N1 isolates had Ser31Asn.



Mechanism of Action of and Development of Resistance to M2 Inhibitors.

In the absence of amantadine, the proton channel mediates an influx of H^+ ions into the infecting virion early in the viral replication cycle, which facilitates the dissociation of the ribonucleoproteins from the virion interior and allows them to be released into the cytoplasm and transported into the cell nucleus. In highly pathogenic avian viruses (H5 and H7), the M2-proton channel protects the hemagglutinin from acid-induced inactivation in the trans-Golgi network during transport to the cell surface. In the presence of amantadine, the channel is blocked and replication is inhibited. The serine at position 31 lies partially in the protein–protein interface and partially in the channel (see inset). Replacement of serine by a larger asparagine leads to the loss of amantadine binding and the restoration of channel function. Depending on the particular amino acid, other mutations at position 26, 27, 30, or 34 may inhibit amantadine binding or allow binding without the loss of ion-channel function. Inset courtesy of Rupert Russell, Phillip Spearpoint, and Alan Hay, National Institute for Medical Research, London.

able in that instance. In addition, in vitro and animal-model studies have shown that combinations of M2 inhibitors and neuraminidase inhibitors have enhanced effects against susceptible viral strains; such combinations warrant further study in the treatment of serious influenza illness due to amantadine-susceptible viruses. Such circumstances highlight the need for rapid methods of ascertaining the susceptibility profiles of epidemic strains — particularly genotypic assays that can be used for quick detection of resistance mutations.[2-4]

One might hope that resistance to neuraminidase inhibitors will not follow the pattern of M2-inhibitor resistance, with widespread community transmission. Depending on the virus type and the assay method used, oseltamivir-resistant variants emerge during therapy in 5 percent to 18 percent of children and less than 1 percent of adults infected with human influenza viruses.[5] In general, the emergence of in vitro resistance to the neuraminidase inhibitors does not correlate with clinical failure in treated immunocompetent patients who are infected by human viruses, although it may in immunocompromised hosts or patients infected with H5N1.[4] Household transmission of resistant viruses has not been observed in controlled studies of neuraminidase inhibitors, and oseltamivir-resistant variants seem much less likely to circulate at the community level than the M2-inhibitor–resistant H3N2 variants have proved to be. Surveillance by the Neuraminidase Inhibitor Susceptibility Network detected a frequency of oseltamivir resistance of less than 0.5 percent among 2287 community isolates collected worldwide during the first three influenza seasons after the drug's introduction (1999 to 2002).[5] During the 2003–2004 influenza season, when Japan had the highest per capita rate of oseltamivir use in the world (approximately 5 percent of the population), only three H3N2 isolates among more than 1100 community influenza isolates contained oseltamivir-resistance mutations. These observations suggest that person-to-person transmission may have occurred at a very low level, although prior drug exposure or newly developed resistance cannot be ruled out. Studies of isolates from the 2004–2005 season, when Japan had even higher levels of oseltamivir use, are in progress.

As for the viruses that constitute pandemic threats, circulating avian H5N1 viruses that have served as the source of human infections have so far remained susceptible to neuraminidase inhibitors.[5] The frequency of emergence of resistance during oseltamivir treatment of patients with H5N1 infection is uncertain, but the His274Tyr mutation conferring high-level oseltamivir resistance was detected in the pharynx of two of eight patients with H5N1 infection (25 percent) and may have been associated with disease progression.[4] However, this mutation leads to reduced replication in animal models of H1N1 and H5N1 infections, and modeling predicts that even if they are only slightly less transmissible than wild-type virus, oseltamivir-resistant variants would be unlikely to spread in the community in spite of high levels of drug use. Moreover, this oseltamivir-resistant variant is fully susceptible to zanamivir,[5] as are several other oseltamivir-resistant viruses. Consequently, although inhaled zanamivir has not yet been studied in human H5N1 disease, it would be a reasonable choice for prophylaxis, especially in persons who have been exposed to oseltamivir-treated patients who might be shedding resistant variants. In order to manage current and future outbreaks of influenza, however, it is essential that we enhance systematic monitoring for the emergence of resistant viruses, rapidly incorporate data on resistance into our treatment strategies, and proceed with development of alternative agents.

Dr. Hayden reports having received lecture fees from Roche, MedImmune, and Biocryst and having served as an unpaid consultant for multiple pharmaceutical companies engaged in influenza antiviral research and for the World Health Organization.

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